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Cover Illustration: A female leatherback turtle *Dermochelys coriacea* emerging on a beach of Amami-oshima Island, Ryukyu Archipelago, Japan. A photograph taken by Yoichi Baba.

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Obituary: Prof. Hajime Fukada, 1913–2002

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Fig. 1. Hajime Fukada around his 50s (*through the courtesy of Tae Fukada*).

Hajime Fukada, Dr. Sci., an honorary member of the Herpetological Society of Japan (HSJ), and Professor Emeritus of Kyoto University of Education, died on 12 March 2002 at the age of 88.

Prof. Fukada was born in Kyoto on 23 August 1913. According to the brief biography in his book “Snake Life History in Kyoto”, he was first attracted to snakes after he caught a Japanese rat snake, *Elaphe climacophora*, when he was in elementary school. But he majored in applied entomology at the Faculty of Agriculture, Kyoto Imperial University. In March, 1937, he graduated the course and became a Master course student in genetics at the Faculty of Science of the same university. He had to interrupt his studies because he was forced to enter army service in January of 1938. He was assigned to the 20th Cavalry Regiment at Kyoto, and passed the examination for the first class cadre candidates in the same year. He was in a candidate course for military officer of meteorology, and finished the program in 1939. He gained the head of his class and received the prize of silver watch from the War Minister. As a military officer of meteorology, he trained noncommissioned officer students and second class cadre candidates. In 1941, he was sent to Pingtung (Pingdong) in southwest Taiwan to serve at the meteorological observatory. He collected some snakes there and kept them in his barracks. Once he was visited by Dr. Kenji Nakamura of Kyoto Imperial University, then a lieutenant, and enjoyed talking about these snakes.

In 1942, he moved to the Philippines by ship and landed on Lingayen. He was appointed an adjutant commander of the 22nd Field Meteorological Battalion, but in September, he was fortunately discharged from military service. In November, he landed in Osaka, and on 12 December 1942, he received demobilization

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orders and returned to the graduate course at Kyoto. In April, 1943, his supervisor Prof. Taku Komai recommended him as a professor of zoology at Ryojun (Lushun) High School (old school system), and on 26 May, just one day after his marriage, he left Kyoto for Ryojun with his bride Tae. At Ryojun, he had to spend more time for military training than zoological class, because he was placed on the reserve list as a lieutenant. However, while teaching horseriding at Shusuishi (Zhoushuizi), he encountered several animals like hedgehogs and kept them as pets at home. Unfortunately, he had little chance to see snakes there.

In August, 1945, the war ended and Ryojun was occupied by the USSR. In October he left Ryojun and moved to Dairen (Dalien) and became a teacher at a newly built Sino-Japanese middle school. At the end of February in 1947, he returned to Japan on a repatriation ship. In July, he got a job as an entomological technician at Otsu Hydroponic Farming Depot of the U. S. Occupation Army. He was interested in the work there and continued it for seven years. At this depot, all successive commanders allowed him to keep snakes in the laboratory and he accumulated much data on the habits, including oviposition, of snakes.

In May of 1954, he got a position of lecturer at Kyoto Gakugei University (now Kyoto University of Education), and began intensive ecological studies of snakes around the paddy fields west of Tanbabashi, Fushimi-ku, in the southern part of the city of Kyoto. He very frequently visited his survey area and did mark-recapture studies on a huge number of snakes. He kept many snakes in the laboratory and recorded data on growth over many years, and steadily published his results in English. In 1955, he was appointed associate professor, and in 1961, he got the degree of Doctor of Science from the Faculty of Science, Kyoto University, under the supervision of Prof. Kenji Nakamura. The title of his thesis was "On the ecology of snakes in the field."

In 1961, he attended a meeting to prepare for the establishment of the Herpetological Society of Japan (HSJ), and in the next year, at the time of the foundation of the society, he became a member. In 1966, he was promoted to full professor and concurrently served as the principal of Momoyama Middle School, attached to the University, for four years. In 1968, he got a grant from the Japanese Ministry of Education and stayed in the USA for three months. During this stay, he met with many herpetologists from around the world and established long-lasting friendships with them.

In 1972, the main office of HSJ moved from Aoyama Gakuin University, Tokyo, to his laboratory at Kyoto University of Education, and he continued to manage the office of the society until 1980. He served as the Secretary of HSJ from 1972 to 1976, and he was an officer of the society from 1974 to 1978. At the same time, he was the Managing Editor from 1977 to 1986. In 1977, he retired from Kyoto University of Education and became an Emeritus Professor. He became the President of the Herpetological Society of Japan in 1978 and kept this position until 1988. Meanwhile, many leading herpetologists, such as Er-mi Zhao, Yehudah Werner, and Kraig Adler, visited his home. He received them cordially and enjoyed talking about herps. Even after he retired from the university, he kept snakes for a long time at home, and liked to show these snakes to his guests.

In 1983, he got a position as professor in biology at Kyoto University of Foreign Languages, and continued teaching until he retired in 1988, when he was decorated with the Third Order of Merit with the Middle Cordon of the Rising Sun. Meanwhile, he had serious problems with his health, and he could not attend the First Sino-Japanese Herpetological Symposium held from August to September 1985 in Guangzhou, in spite of his great efforts to coordinate the Japanese delegates. In 1986, he had surgery for the tip of the pancreas, the spleen, and the left kidney at Kyoto University Hospital. In 1988, he again received an operation for exfoliated ureter, and in 1989 for ureter stoma. In 1988, he was elected a honorary member of HSJ. In spite of his poor health, he continued his study, and presented an oral paper at the Second Japan-China Herpetological symposium held in July at Kyoto University. At home and in hospital, he steadily continued to compile field and laboratory data he had accumulated for over 40 years, and finally completed the publication of his monumental life work, Snake Life History in Kyoto, in 1992. In October of the same year, he attended the 31st annual meeting of HSJ at Kyoto University and presented a paper on the recapture of snakes.

In 1993, he had to stay in the hospital for two months for Hodgkin's disease, and in 1994, he began to receive kidney dialysis. Since then, he entered the hospital for a short time every year, and received dialysis treatments twice a week. Even in this adverse situation, he never forgot herpetology, and in November 1997, he presented a poster paper on captive longevity of snakes at the 36th annual meeting of HSJ at Kyoto University. Unfortunately, this turned out to be his last speech at HSJ annual meetings. In 2000, he again entered the hospital for one month because Hodgkin's disease recurred. From 2001, he had to receive dialysis three times a week. I visited the Professor's home on 3 March 2002 for a short while and took photographs of him. He seemed to be not so bad and enjoyed talking about HSJ, but this was my last chance to meet him. Nine days later, he died at the NTT Hospital, Minami-ku, Kyoto, because of cardiac failure. He was decorated in recognition of his brilliant services in scientific education on the same day.

Because Prof. Fukada belonged to a university of education, where there was no graduate course, he had few pupils who became professional herpetologists. However, he will surely be remembered by the works he left behind and for his great contributions to the advancement of Japanese herpetology.

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Male Courtship Behavior of *Tylototriton* (*Echinotriton*) *andersoni* Boulenger under Laboratory Conditions

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Abstract: We observed male courtship behavior of *Tylototriton* (*Echinotriton*) *andersoni* in the laboratory. In some males, the cloaca swelled and became wet with mucous secretions from December to May. A male in this condition crept around a female, sniffing around her body and drawing a thread of mucus from his cloaca, so that the female was surrounded by spiderweb-like string of mucous attached to the substrate. The male then deposited a spermatophore by rubbing his cloaca against the substratum while swaying his body to and fro. The male behaved similarly towards females from different localities, though we could not observe any female reaction to the male. Under a phase-contrast microscope, the gross morphology of sperms from a male from Amamioshima Island was similar to that reported for males from Okinawajima.

Key words: Male courtship behavior; *Tylototriton* (*Echinotriton*) *andersoni*; Spermatophore deposition; Sperm morphology; Amamioshima; Tokunoshima; Okinawajima

INTRODUCTION

Since its original description by Boulenger (1892) on the basis of a specimen from Okinawajima, the Anderson's alligator newt, *Tylototriton andersoni*, has been viewed as a rare (Tago, 1931), relict species. Reports on this species were thus mostly limited to collection records or brief notes on a few biological aspects for a very long time (Ichikawa, 1941; Momma and Makino, 1941; Koba, 1955, 1956). Sato (1943) reviewed biological data accumulated by that time, but details of distribution, precise habitats, and breeding ecology were totally unknown.

One of us (Utsunomiya, 1973) first observed in the laboratory that the female of this species lays eggs in the soil, unlike other Japanese urodeles. Subsequently, Utsunomiya et al. (1978) and Matayoshi et al. (1977, 1978) reported detailed distribution and several ecological aspects, especially egg-laying habits in natural habitats, of this species. Through these more recent studies, a gross outline of the breeding habits of this species was provided. For example, it was shown that this newt never enters the water after metamorphosis and lays eggs in the soil under leaf litter that could be kept moist during incubation. The oviposition site is located near the water, but is never flooded. The female rolls her eggs with her snout and buries them deep in the soil, sometimes as deep as eight cm. It is also known that the male is not present at oviposition, and that the female lays fertilized eggs, even after isolation from males for several months. Actually, males rarely visit the breeding sites, as shown by the female-biased sex ratio at the sites (15 males per 237 females: Tanaka, 1994).

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As described below, we found spermatophores deposited in the laboratory more than twenty years ago (Utsunomiya, 1982). It was puzzling, however, how the male deposits and transfers his spermatophores to females without entering the water, unlike many other newts that are aquatic at least in the breeding season (Salthe, 1967). In this article, we summarize male courtship behavior observed in the laboratory, together with gross morphology of the spermatophore. Part of the observations described below have been briefly reported (Utsunomiya, 1982) or cited as a personal communication (Sparreboom et al., 2001).

MATERIALS AND METHODS

Generic classifications of the Oriental alligator newts (*Tylototriton* Anderson, 1871, sensu lato) differ from one author to another. Current herpetologists tend to follow Nussbaum and Brodie's (1982) idea of assigning several species including *andersoni* to a separate genus *Echinotriton* Nussbaum and Brodie, 1982. However, because representatives of *Tylototriton* and *Echinotriton* sensu Nussbaum and Brodie (1982) are genetically very close (Hayashi and Matsui, 1989), the validity of *Echinotriton* as a full genus needs further taxonomic studies. We herewith treat *Echinotriton* as a subgenus of *Tylototriton*.

Nine specimens of *T.* (*E.*) *andersoni* were used for behavioral and spermatic observations: one male collected at Naze-shi, Amami-oshima Island, on 12 April 1980; one female also from Naze-shi on 13 April 1980; two males and one female from Amagi-cho, Tokunoshima Island, on 6 April 1972; one female also from Amagi-cho on 4 January 1981; three females collected as eggs on 8 February 1978 at Gushikawa-shi, Okinawa-jima Island, reared in the laboratory, and attained sexual maturity within four years. The population from Okinawa Prefecture has been protected by the prefectural government as a natural monument since 9 November 1978.

Individuals were reared at the room temperature and under natural light conditions in Hiroshima, Honshu, Japan, except for the winter season when the air temperature was kept at 15 C. Each terrarium used measured 36×22×25 cm and one-third each of the bottom was covered with shallow water, pebbles, and sand. Pieces of slate were laid on the pebbles for shelters, and leaf litter from the natural habitat was placed on the sand (Utsunomiya, 1973).

We periodically checked each individual, and when some of them showed notable bahaviors, we made intensive observations. To examine sperms, we took male cloacal secretions or the white part of deposited spermatophores, smeared them on glass slides, and examined the slides with a phase contrast microscope. The male secretions were obtained by gently pressing his belly. We also examined these slides with a light microscope after fixig them with either aceto-orcein or Navasin's solution and staining them with Heidenhain's hematoxylin.

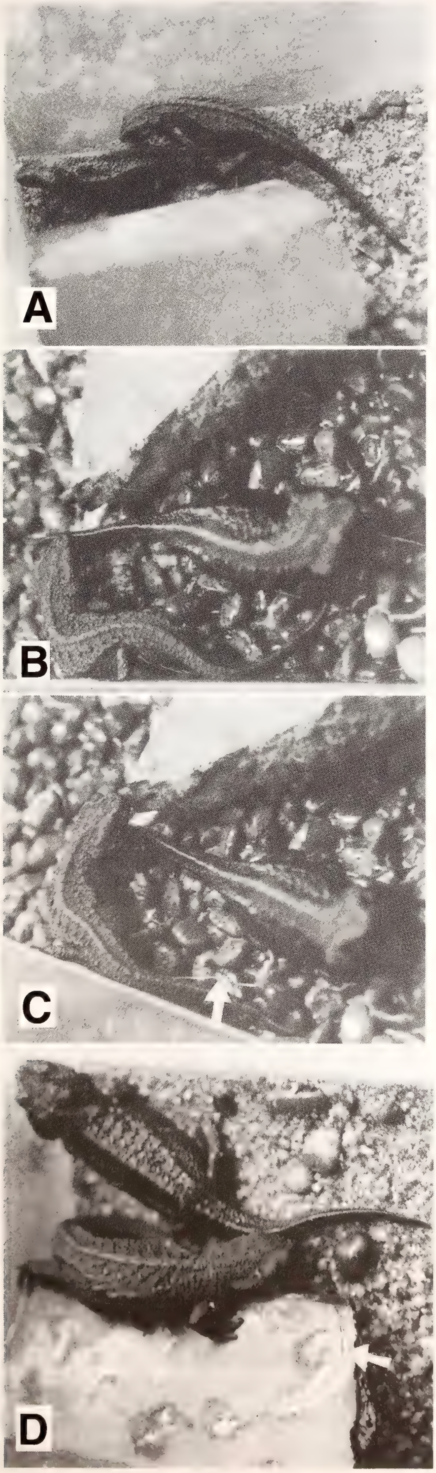
RESULTS

Morphological change in the breeding season

Three adults from Tokunoshima could not be sexed at the time of collection on 6 April 1972, but at the time of observation on 14 February 1973, two of them had a swollen cloaca. The remaining one that did not have a swollen cloaca proved to be a female because it laid fertile eggs on 9 May 1973. Thus, the possession of a swollen cloaca by the two individuals also proved to be an indication that they were males.

Male courtship behavior

On 16 April 1982, we found the cloaca of the male from Amamioshima to be swollen and wet with secretions. Because the female from the same island kept with him showed no response, we put him in another terrarium containing a female from Okinawajima. Soon after this manipulation, the male tried to sniff at the female with his snout close to her body (Fig. 1A, B). The female also once reacted to the male in such a way as to seem to be trying to sniff at him, but soon stopped. The male sniffed at her in the order of head, belly, cloaca, and tail tip, and repeated this behavior several times. The female, however, showed no response to his behavior.



Meanwhile, the male pressed his cloaca on a substrate pebble and secreted transparent mucus. Then he moved forward for 5–10 cm and again pressed his cloaca to another pebble. By this action, the mucus was extended like a string (Fig. 1C, D). The male continued this action around the female, keeping his snout close to her, and the string of mucus was stretched around the female like a spider's web. Unfortunately, most portions of the mucous strings soon disappeared on the pebbles that were always kept wet to maintain moisture in the cage.

After continuing this behavior for about three minutes, the male placed his cloaca on a pebble, swaying his waist. Then he moved his body to and fro and slowly lifted his pelvic region from the pebble. Below his cloaca, a conical spermatophore could be seen tightly attached to the pebble. There were white portions in and at the top of the otherwise transparent spermatophore. After this first spermatophore deposition, the male resumed pursuing the female, pulling the mucous strings. About ten minutes later, the female hid under a slate, and we had to cease observation.

We put the active male into another cage containing a female from Tokunoshima, but the male did not react toward the female during ten minutes of observation.

Then we put the male into still another cage containing the two remaining females from

FIG. 1. A male *Tylototriton* (*Echinotriton*) *andersoni* sniffing at a female (A, B) and a string of mucus from the male cloaca (white arrow, C, D).

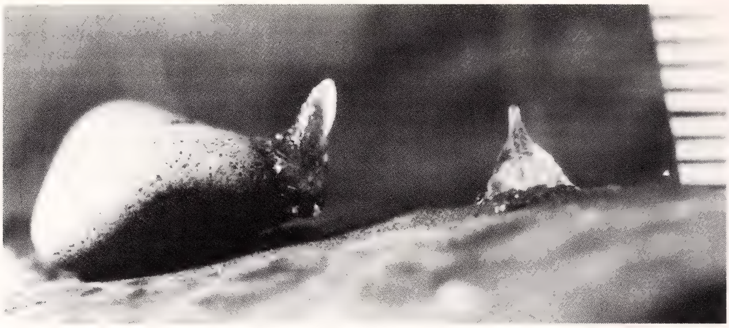


FIG. 2. Two spermatophores of *Tylototriton* (*Echinotriton*) *andersoni* deposited on a pebble and a shelter slate. Scale indicates 1 mm.

Okinawajima that were smaller sibs of the female mentioned above. The male soon reacted to one of them and resumed the same behavior as described above. He pressed his cloaca to a shelter slate and left a clear mucous string on it. When the forelimb of the female became tangled in the mucus, she vigorously tried to remove it by rubbing her hand in the substrate sand and tried to escape from the male. Notwithstanding her action, the male persistently tried to move around the female drugging mucous strings from his cloaca. We failed to continue observation, but, ten minutes later, we found two spermatophores, one on a pebble and another on the slate. They were connected by mucous strings and thus seemed to have been deposited one after the other (Fig. 2).

Spermatophore morphology

On 10 December 1981, we found two spermatophores attached to the surface of a slate shelter in a cage containing the male and the female from Amamioshima. Both spermatophores were nearly conical in shape (Fig. 2), transparent like crystal, and partially spotted with white. Their material was hard but somewhat jelly-like.

On 23 January 1982, the male from Amamioshima again deposited a spermatophore on a slate. It was 4.2×4.0 mm in base diameter and 7.1 mm in height. As in the previous case, the spermatophore was hard, jelly-like, and transparent with white portions on top and inside.

Sperm morphology

When one female from Tokunoshima laid eggs on 9 May 1973, we took them out of the soil and washed them in a small amount of water. We observed the water thus obtained under a light microscope, but could not find any sperms.

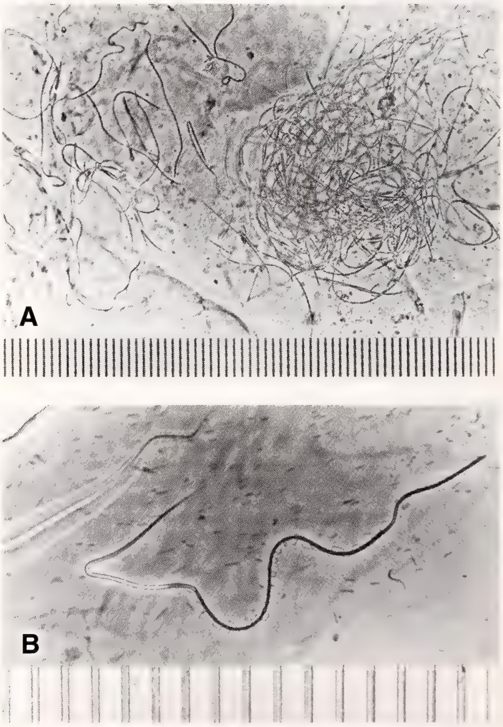


FIG. 3. Clumps of sperms of *Tylototriton* (*Echinotriton*) *andersoni* in the spermatophore (A) and a spermatozoon observed under a phase-contarst microscope (B). Scale indicates 10 μm.

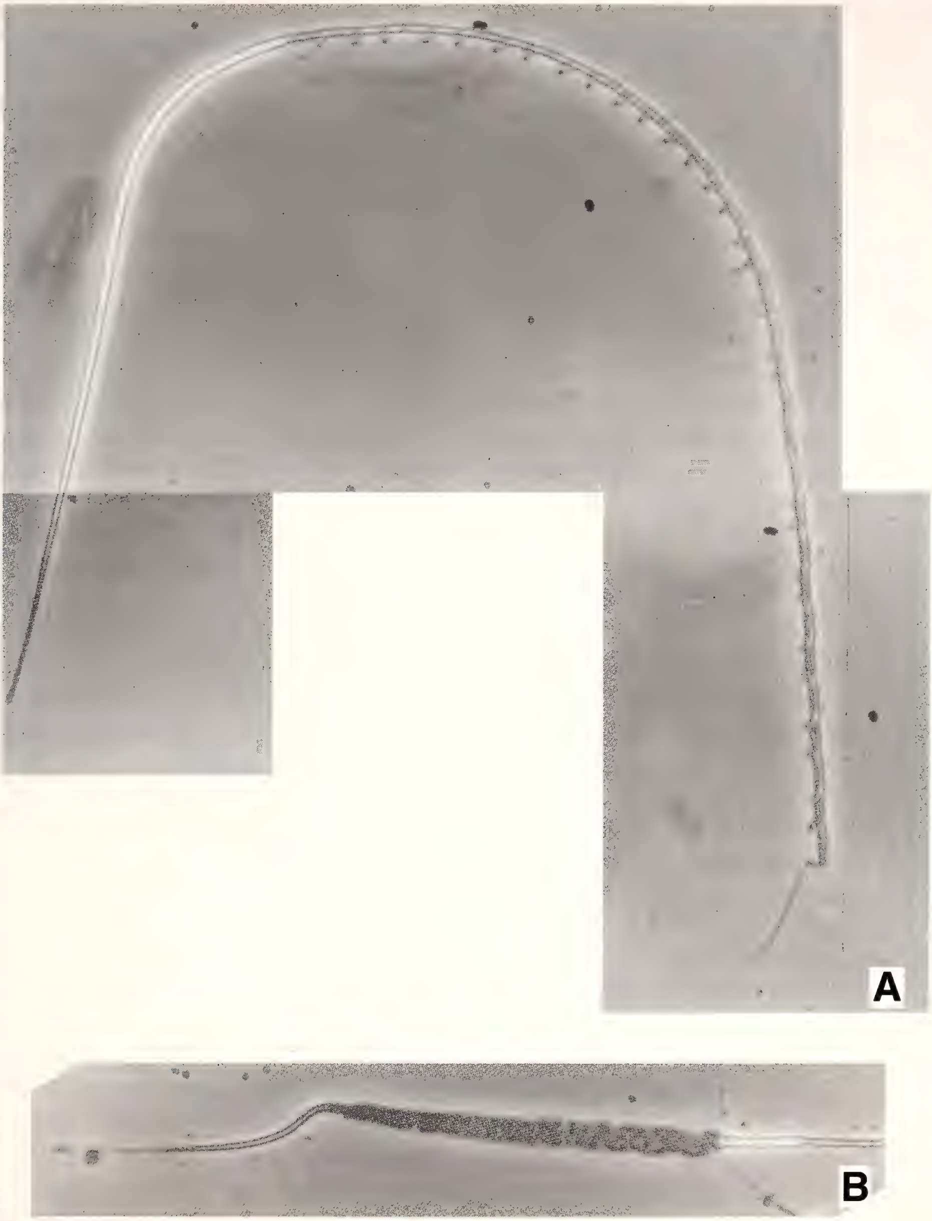


FIG. 4. A spermatozoon of *Tylototriton* (*Echinotriton*) *andersoni* observed under a phase-contarst microscope (A) and the head and neck region observed under a light microscope after staining with aceto-orcein (B). ×ca. 1300.

For the spermatophores deposited on 10 December 1981 by the male from Amami-oshima, we continued observation for ten days, in order to witness the way the female took up these spermatophores. Unfortunately, however, the female showed no particular response, and the spermatophores became gradually smaller day by day. Therefore, we gave up further observation and observed these spermatophores under a phase-contrast microscope. In the white portion of the spermatophore, we could observe numerous sperms moving slowly, forming a clump (Fig. 3A).

We failed to measure the size of a spermatozoon exactly, but its total length was approximately 250 μm (Figs. 3B, 4A). In a spermatozoon, in order, the acrosome, the head proper, the neck piece, the axial rod, and the end piece could be identified (Fig. 4A). The acrosome and the neck piece were not always clearly distinguishable from the head proper under the phase contrast microscope (Fig. 4A, but see Fig. 3B), but the neck piece was clearly recognized by its thickness and chromatic nature when stained with aceto orcein (Fig. 4B). The end piece was relatively long, and was formed by a posterior extension of the flagellum, and was free from the axial rod (Fig. 4A).

DISCUSSION

Newts of the family Salamandridae are highly deiversified in their reproductive habits and quite a few studies have been made on their courtship behavior (e.g., Salthe, 1967; Halliday, 1977). Males of some genera (e.g., *Cynops, Triturus*) court by tail-fanning in front of their mates (Kawamura and Sawada, 1959; Halliday, 1977), while those of other genera (e.g., *Pleurodeles, Salamandra*) clasp females before spermatophore deposition (Arnold, 1977). Most members of this family generally breed in the water, and of the genus *Tylototriton*, all four species of the subgenus *Tylototriton*, *T.* (*T.*) *taliangensis*, *T.* (*T.*) *kweichowensis*, *T.* (*T.*) *shanjing* (Fei et al. 1999: *T.* (*T.*) *shanjing* treated as *T.* (*T.*) *verrucosus*), and *T.* (*T.*) *verrucosus*, also breed in the water (Annandale, 1908; Smith, 1924; Fei et al. 1999).

On the other hand, all members of the subgenus *Echinotriton*, *T.* (*E.*) *andersoni*, *T.* (*E.*) *chinhaiensis*, and *T.* (*E.*) *asperrimus*, lay eggs on land (Utsunomiya, 1973; Fei et al. 1999). The paucity of detailed relevant information on these terrestrial breeders, however, has been an obstacle to inferring evolutionary trends in the courtship behavior of the Salamandridae (Sparreboom et al., 2001).

Because *T.* (*E.*) *andersoni* never enters the water after metamorphosis, spermatophore transfer in this species is enigmatic. As shown above, our observations in the laboratory were successful in elucidating the male behavior for depositing spermatophores, but we failed to detect the way the female receives spermatophores. It is probable that the females we used were not ready for receiving sperms due to their age (too old or too young). Also, differences in locality between the females and the male might have prevented acceptance of the spermatophores by the females, considering the substantial between-island genetic and morphological variation in *T.* (*E.*) *andersoni* (Hayashi et al., 1992). However, the male indiscriminately exhibited courtship behavior toward females from different localities.

Sparreboom et al. (2001), on the basis of captive observations, reported mating behavior of *T.* (*E.*) *chinhaiensis*, which was basically similar to that reported here for *T.* (*E.*) *andersoni* with respect to the male behavior up to the stage of spermatophore deposition. In both species, for example, the male, after sniffing the female, marks a trail around her by excreting mucous from his cloaca, and finally deposits the spermatophore without clasping her. Like male *T.* (*E.*) *andersoni*, male *T.* (*E.*) *chinhaiensis* may deposit several spermatophores in one evening (Sparreboom et al., 2001).

Sparreboom et al. (2001) observed the trail-marking by mucous excretion from the male's cloaca in *T.* (*E.*) *chinhaiensis*, and assumed the behavior to be a remarkable feature of this species. They also surmised that this trail, presumably serving as orientation for the female through olfactory or tactic stimuli, has evolved as an adaptation to underground mating. But we are dubious of this view because the male *T.* (*E.*) *andersoni* observed by us marked a trail only on pebbles and slates, never on the surrounding soil. We suspect the spiderweb-like threads to be an apomorphy of *Echinotriton*, although this view needs substantial verification on the basis of observations on the other species of this subgenus.

In this context, the behavior of *T.* (*E.*) *asperrimus* should be examined, because its allocation to the subgenus *Echinotriton* is questionable (Nussbaum et al., 1995).

In our observations, females showed no particular responses to the male's courtship behavior including rejection or unresponsiveness, such as the snout-kick which Sparreboom et al. (2001) found in *T.* (*E.*) *chinhaiensis*. In that species, the female, when pairing with the male, follows him on an ellipsoid track. After deposition of a spermatophore by the male, the female is lead over it by aligning her body with the male. Finally the female shifts her pelvic region and traces the spermatophore with her cloaca (Sparreboom et al., 2001). Sparreboom et al. (2001) noted that the female's success or failure in receiving the sperm mass was ascertained by the removal of the white sperm cap from the spermatophore base, but they did not make it clear whether or not the sperm cap had been absorbed in the female's cloaca.

Most probably, a succession of similar behaviors enables the female of *T.* (*E.*) *andersoni* to receive the spermatophore. Future detailed studies of female behavior in this species, including her treatment of the sperm cap, which Sparreboom et al. (2001) failed to ascertain in *T.* (*E.*) *chinhaiensis*, are strongly desired both in the laboratory and in the field.

As briefly mentioned by Sparreboom et al. (2001) on the basis of information from one of us (Utsunomiya, pers. comm., 2000), the spermatophore of *T.* (*E.*) *andersoni* (4.2×4.0 mm in base diameter and 7.1 mm in height) was much larger than that of *T. chinhaiensis* (2.5–3 mm both in base diameter and in height), and this difference, approximately 4–7 times in volume, might lead to a difference in the frequency of spermatophore deposition. The spermatozoon of *T.* (*E.*) *andersonii* from Okinawajima was described in detail by Kuramoto and Tanaka (1997). The morphology of sperms from the Amamioshima male examined here basically fits their description well. All major characteristics observed in the spermatozoa of *T.* (*E.*) *andersonii* (e.g., much smaller size [c.a. 250 μm] than that of *Cynops pyrrhogaster* [419 μm: Mori, 1936], possession of a flagellum end which is free from the axial rod, thus forming a long end piece) agree well with those reported by Kuramoto and Tanaka (1997). We could not consistently distinguish the acrosome and the neck piece from the head proper in our observations with a phase-contrast microscope. Kuramoto and Tanaka (1997) similarly could not clearly distinguish them from head proper in their SEM observations. This indicates the necessity of employing more than one method for correct observation of sperm morphology.

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Female Size-specific Clutch Parameters of Two Closely Related Stream-breeding Frogs, *Rana sakuraii* and *R. tagoi tagoi*: Female Size-independent and Size-dependent Egg Sizes

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Abstract: Clutch parameters, such as clutch size, egg size, and clutch volume were studied in relation to female body size for two closely related species of stream-breeding frogs, *Rana sakuraii* and *R. tagoi tagoi*, in the Kanto district of Honshu, Japan. Two populations were studied for each species. Mean clutch size (approximately 130) did not show significant interspecific or intraspecific variation. In contrast, mean egg size differed between species or conspecific populations. *Rana sakuraii* laid larger eggs than any other anuran species in Japan (3.20 and 3.36 mm in mean diameters of two populations), but the eggs of *R. t. tagoi* were slightly smaller (2.79 and 2.94 mm). We could not detect any significant effect of age on clutch parameters in *R. t. tagoi*, after adjusting for female body size. We reviewed data on the relationship between female size and egg size in amphibian species, and found that most (71%) of the species or populations so far studied show a significantly positive correlation between these parameters. In the present study, no significant correlation was recognized between female size and egg size in *R. sakuraii*, but they were significantly positively correlated in *R. t. tagoi*. The evolutionary significance of variation in the relationship between these parameters is discussed.

Key words: *Rana sakuraii*; *Rana tagoi*; Clutch parameters; Age; Relationship between female size and egg size

INTRODUCTION

Egg size is usually one of the most important fitness components in species with no parental care, since this parameter may strongly affect growth and survival of hatchlings and clutch size in females. Recent studies showed that in a variety of animal groups the egg size varies not only among conspecific populations or different species, but also among females belonging to the same population or even within the same individual (see Roff, 1992). For elucidation of ultimate factors determining the egg size, considerations regarding patterns of inter- and intrapopulational variations in this parameter and their proximal causes are essential.

Egg size variation has been documented for

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some Japanese amphibians, such as *Bufo japonicus* (Matsui, 1989), *Hynobius nigrescens* (Takahashi and Iwasawa, 1988), and *H. tokyoensis* (Kusano and Hayase, 1990). Nevertheless, little information is yet available regarding the quantitative aspect of egg size and other clutch parameters for most Japanese amphibians.

In many populations or species, egg size is correlated with female body size: larger females produce larger eggs (see Roff, 1992). Therefore, when we analyze egg size variation, the relationship between female body size and egg size must be taken into consideration. However, the correlation just noted is not always the case: for example, in some amphibian populations or species, egg size does not depend on female size (e.g., Howard, 1978; Kuramoto, 1978).

Rana sakuraii has the unique ecological trait of breeding in streams (Matsui and Matsui, 1990). Stream breeders are a minor group among Japanese anurans: of the 38 anuran species found in Japan, only seven (*Buergeria buergeri*, *Bufo torrenticola*, four species of the *R. narina* complex, and this species) are typical stream breeders (Maeda and Matsui, 1999). Lotic environments in streams differ physically and ecologically from lentic ones such as those in ponds, and are usually very severe for both breeding adults and larvae. Stream breeders, therefore, must have developed unique morphological, reproductive, and life history traits. The way by which these anurans breed successfully in lotic environments is an interesting issue. At present, however, we have rather little information on stream breeders as compared to pond breeders. We believe that elucidation of reproductive ecology of stream breeders would provide valuable information for better understanding of the evolution of reproductive strategies in anurans.

In the present paper, we examine clutch parameters in relation to female body size in *R. sakuraii* and its close relative, *R. tagoi tagoi*, in the Kanto district. We also discuss ecological and evolutionary factors affecting relative egg size in these species.

MATERIALS AND METHODS

Study site

The breeding populations of *R. sakuraii* and *R. t. tagoi* were studied at five sites: Bonbori, Takigahara, Kusagyu, Ichinosawa, and Kamiange (Fig. 1). The study site at Bonbori is an upper tributary of the Bonbori River (35°42'N, 139°11'E) in Akiruno-shi, Tokyo, at an altitude of 380 m. This stream is located in a mountainous region covered with planted coniferous forests of *Cryptomeria japonica* and *Chamaecyparis obtusa* and secondary deciduous forest. At this site, the stream was about 3 m in maximum width and 50 cm in maximum depth. We collected breeding adults of *R. sakuraii* by hand or using drift nets during the breeding seasons (February–early March) of 1986–1988 (see Kusano and Fukuyama, 1989). We also collected *R. sakuraii* from two other sites in Tochigi Prefecture: Takigahara and Kusagyu. The study site at Takigahara is an upper tributary of the Kurokawa River (36°42'N, 139°35'E) in Nikko-shi, at an altitude of 530 m. The other study site at Kusagyu is an upper

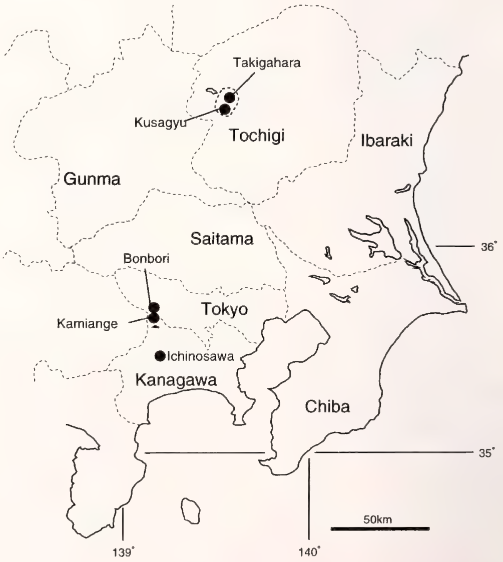

FIG. 1. Locations of study sites in the Kanto district, Honshu, Japan.

tributary of the Oashigawa River (36°39'N, 139°34'E) in Kanuma-shi at an altitude of 440 m. Both of these streams are also located in a mountainous region, covered with planted coniferous forests of *C. japonica*, and were about 3–5 m in maximum width and 50 cm in maximum depth. Breeding adults were collected by hand during late February–late March of 1999–2000.

The breeding adults of *R. t. tagoi* were collected from Ichinosawa in Kiyokawa-mura, Kanagawa Prefecture, and from Kamiange in Hachioji-shi, Tokyo. The study site at Ichinosawa is a small stream near the headspring of an upper tributary of the Nunokawa River (35°28'N, 139°13'E), at an altitude of 460 m. The stream is located in a mountainous region chiefly covered with coniferous forests of *Abies firma* and *Tsuga sieboldii*. At this site, the stream was about 1 m in maximum width and 20 cm in maximum depth. The other study site at Kamiange is a seepage area on the banks of an upper tributary of the Kitaasakawa River (35°39'N, 139°11'E) at an elevation of 460 m. The surrounding region is covered with planted coniferous forests of *C. japonica* and *C. obtusa* and secondary deciduous forest. We collected breeding adults by hand during April of 1987–1990 (see Kusano et al., 1995a).

Measurements of clutch parameters

Each gravid female collected was measured for snout-vent length (SVL) to the nearest 0.5 mm with a sliding caliper, and was placed with a male in an individual plastic container (15 cm in diameter and 9 cm in depth) with aged tap water (4 cm in depth). Most of the *R. t. tagoi* females deposited eggs by the next morning, but *R. sakuraii* required several days until spawning (Kusano and Fukuyama, 1987). Eggs of each clutch were separately counted to determine clutch size. A sample of ten (for Tochigi populations) or 20 eggs (for the others) was selected at random from each clutch, and the diameter of each egg was measured to the nearest 0.02 mm with an ocular micrometer attached to a binocular microscope. The developmental stage of eggs (Gosner, 1960) was also recorded at the time of measurement. Mean egg diameter was then calculated for each sample as a representative value for each clutch. The volume of each clutch was calculated from the mean egg diameter (d) and the clutch size (n) as $(1/6)n\pi d^3$, assuming that the eggs were invariably spherical.

Estimating initial egg size

Many eggs were first measured at stages beyond the first cleavage. Because amphibian eggs are known to change in size during early development (Kaplan, 1979; King and Kaplan, 1997), we needed to estimate initial sizes for eggs that were measured after some development. For such estimations, 20 uncleaved eggs were collected from a newly deposited clutch of female *R. t. tagoi* from Kamiange in April 1988. Eggs were measured immediately after being laid, and then were placed in a petri dish containing aged tap water, where they were kept at 22C. At various stages of their early development (up to stage 10), the eggs were staged and measured to record changes in diameter.

Age determination

Toes were clipped off from part of the *R. t. tagoi* females captured and were stored in separate vials containing 10% buffered formalin. By counting year rings in the hematoxylin-stained crosssections of these phalanges (see Kusano et al., 1995a, b), age was determined for each individual to examine its effects on clutch parameters.

Statistical analysis

Statistical analyses were performed with the Statistical Analyses Software version 6.12 (SAS Institute Inc., 1990). Differences of clutch parameters were examined among populations using a one-way analysis of variance (ANOVA). The relationships between female body size and clutch parameters were analyzed using simple regression equations. The regression equation between body size and clutch volume was calculated after both variables were log-transformed. These regressions were compared among populations by an analysis of covariance (ANCOVA). The significance level in all tests was $P<0.05$. Multiple comparisons for means were made following the procedure of the Tukey test (Zar, 1996).

RESULTS

Estimating initial egg size

The eggs of *R. t. tagoi* used in the experiment ranged from 2.70 to 2.90 mm ($\bar{x}=2.81$, SD=0.069) in diameter at the initial uncleaved stage. Egg diameter increased with the progress of development to 3.01 mm in mean (SD=0.063) (Fig. 2). The rate of increase was marginal (2.0%) up to stage 7, but then became more rapid. The mean diameter of eggs at stage 10 was larger than that at the uncleaved initial stage by 7.1%. Using the data depicted in Fig. 2, diameters of all eggs measured were converted to those at the initial uncleaved stage.

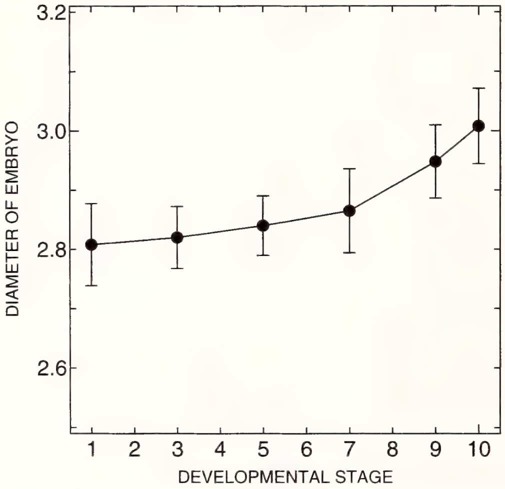

FIG. 2. The relationship between developmental stages up to the start of gastrulation and ovum diameter ($\bar{x}$±SD, n=20) in *Rana tagoi tagoi*.

Body size and clutch parameters

A total of 41 gravid females of *R. sakuraii* were collected from Bonbori, and 12 and three females were from Takigahara and Kusagyu, respectively. Because the latter two study sites are close to each other (only 5 km apart: Fig. 1) and values for clutch parameters in the three females from Kusagyu were all within the range of corresponding values in the Takigahara females, data for clutch parameters from the two sites were pooled as those for a Tochigi population.

A total of 34 and 20 females of *R. t. tagoi* were collected from Ichinosawa and Kamiange, respectively. We could not determine clutch sizes for parts of these samples, since some females apparently failed to deposit complete clutches.

Clutch parameters of females representing the four populations of the two species are shown in Table 1. Clutch size varied from 53 to 186 in *R. sakuraii*, and from 63 to 200 in *R. t. tagoi*. The mean clutch size did not differ significantly among the four populations. By contrast, significant differences were detected in egg size and clutch volume among those populations (Table 1). Egg size ranged from

TABLE 1. Body size and clutch parameters of populations of *Rana sakuraii* and *R. tagoi tagoi*.

	R. sakuraii				*R. t. tagoi*					
	Bonbori		Tochigi		Ichinosawa		Kamiange		ANOVA	
Variables	$\bar{x}$±SD	n	$\bar{x}$±SD	n	$\bar{x}$±SD	n	$\bar{x}$±SD	n	F	P
Snout-vent length (mm)	48.9±3.1	41	51.9±2.7	15	44.0±2.5	34	42.1±3.4	20	48.77	<0.0001
Clutch size	126.4±29.7	41	131.1±27.5	14	128.5±28.2	34	130.5±31.1	18	0.13	0.9406
Egg size (mm)	3.20±0.10	41	3.36±0.15	15	2.94±0.09	34	2.79±0.13	20	107.63	<0.0001
Clutch volume (mm^3)	2169±529	41	2624±665	14	1713±424	34	1518±464	18	17.49	<0.0001

2.98 to 3.57 mm in *R. sakuraii* at Bonbori, from 3.08 to 3.58 mm in this species at Tochigi, from 2.73 to 3.11 mm in *R. t. tagoi* at Ichinosawa, and from 2.53 to 2.95 mm in this species at Kamiange. In the Tukey multiple range test, the mean egg size differed significantly, not only between the two species, but also between the two populations of each species.

Body size also differed significantly between the two species: *R. sakuraii* was, on average, at least 10% larger than *R. t. tagoi* (Table 1). In addition, SVL differed significantly between the two populations of *R. sakuraii*. Since clutch parameters of amphibian species, such as egg size and clutch size, often show significant correlations with female body size (e.g., Kuramoto, 1978; Kaplan, 1980), effects of variation in female size should always be considered when comparing those clutch parameters between populations.

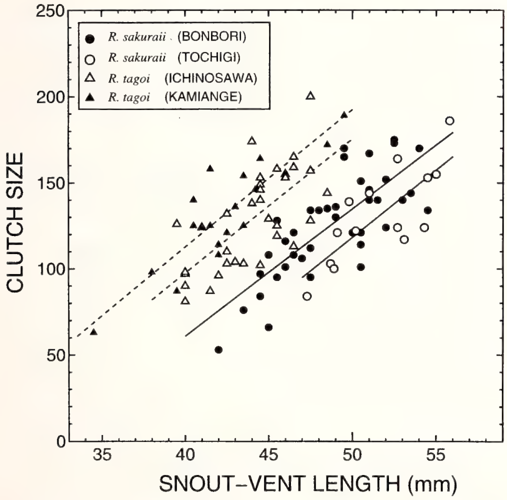

FIG. 3. Relationship between female body size and clutch size in four populations of *Rana sakuraii* and *R. t. tagoi*. Regressions of the four populations differed significantly in elevation. Solid lines denote regressions for Bonbori ($Y=7.40X-235$, $r=0.774$, $n=41$: above) and Tochigi ($Y=7.82X-273$, $r=0.771$, $n=14$: below) populations of *R. sakuraii*. Dashed lines denote regressions for Kamiange ($Y=7.99X-207$, $r=0.879$, $n=18$: above) and Ichinosawa ($Y=7.79\ X-214$, $r=0.690$, $n=34$: below) populations of *R. t. tagoi*.

Females of both species showed a significant positive correlation between clutch size and SVL (Fig. 3). The ANCOVA showed that *R. sakuraii* deposited a fewer number of eggs for body size than *R. t. tagoi*: the adjusted means were 108.8, 92.1, 148.3, and 163.5 at Bonbori, Tochigi, Ichinosawa, and Kamiange, respectively. The differences of adjusted means were all statistically significant among the four populations (Tukey test, $P<0.0001$ between both species, and $P=0.0347$ between Bonbori and Tochigi, $P=0.0363$ between Ichinosawa and Kamiange).

For *R. t. tagoi*, egg size was also significantly correlated with female SVL: larger females deposited larger eggs than smaller ones (Fig. 4; $r=0.636$, for Ichinosawa, $r=0.774$ for Kamiange, $P<0.0001$ in both). The regressions differed significantly in elevation between the two populations, meaning that similar-sized females deposited larger eggs at Ichinosawa than at Kaminage (ANCOVA, $F=17.75$, $P<0.0001$). By contrast, no significant relationship was detected for the two populations of *R. sakuraii*

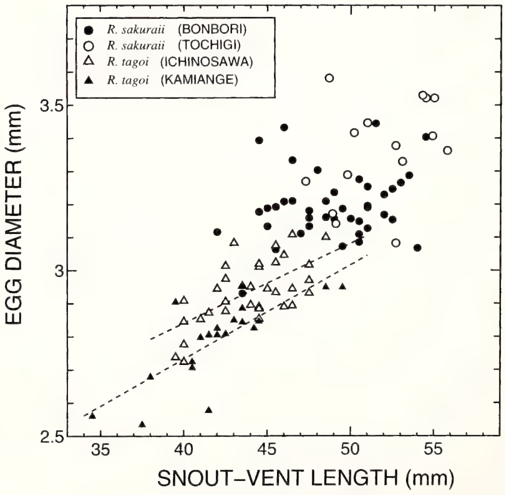

FIG. 4. Relationship between female body size and egg size in four populations of *Rana sakuraii* and *R. t. tagoi*. No significant relationships were recognized in either population (Bonbori and Tochigi) of *R. sakuraii*. Dashed lines denote regressions for Ichinosawa ($Y=0.0241X+1.876$, $r=0.636$, $n=34$: above) and Kamiange ($Y=0.0283X+1.600$, $r=0.774$, $n=20$: below) populations of *R. t. tagoi*.

($r=0.198$, $P=0.215$ at Bonbori; $r=0.362$, $P=0.185$ at Tochigi). All females of *R. sakuraii* but one deposited eggs of more than 3 mm in diameter, and most plots of *R. sakuraii* were located above those of *R. t. tagoi* (Fig. 4). These results indicate that the egg size of *R. sakuraii* was larger than that of *R. t. tagoi*, even if adjusted for body size, and that the mean egg size of the *R. sakuraii* population was significantly larger at Tochigi than at Bonbori ($t=4.471$, $P<0.0001$).

The regression of clutch volume against female SVL did not differ significantly between populations of *R. sakuraii* or of *R. t. tagoi* (Tukey test, $P>0.97$ in both), but differed significantly in elevation between populations of *R. sakuraii* and *R. t. tagoi* ($P<0.025$ in all combinations). The adjusted means of the clutch volumes were 1727, 1694, 2041, and 2080 mm^3 for the Bonbori, Tochigi, Ichinosawa, and Kamiange populations, respectively (Fig. 5). Clutch volume of *R. sakuraii* was, therefore, approximately 17% smaller than that of *R. t. tagoi* when compared between similar-sized females.

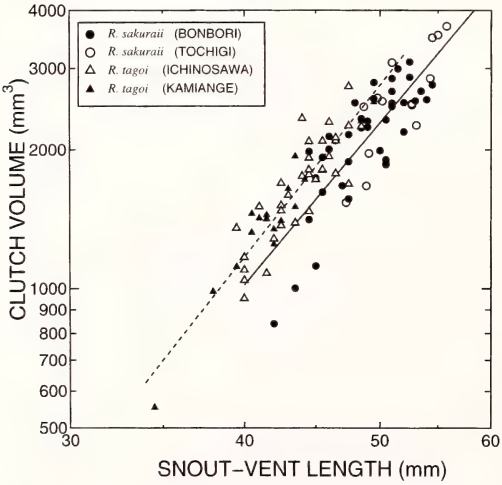

FIG. 5. Relationship between female body size and clutch volume. The log-log regression did not differ significantly between two populations in each species, and a single regression equation was thus calculated from combined conspecific data sets. Solid and dashed lines indicate regressions of *R. sakuraii* ($\log Y=3.627\log X-2.802$, $r=0.82$, $n=55$) and *R. t. tagoi* ($\log Y=3.848\log X-3.097$, $r=0.90$, $n=52$), respectively.

Age and clutch parameters

A total of 26 females of *R. t. tagoi* (18 and eight from Ichinosawa and Kamiange, respectively) was aged by skeletochronology using phalanges. Their ages ranged from two to four years (see also Kusano et al., 1995a). Because clutch parameters were closely related to body size as mentioned above (Figs. 3–5), it was necessary to examine the relationship between age and clutch parameters after removing the effect of female SVL. Relative egg and clutch sizes were, therefore, calculated as residuals from the regressions of these parameters against SVL (Figs. 3 and 4). We could not find any clear trends in the relationship between age and these parameters (Fig. 6). Partial correlation coefficients were also calculated to eliminate any effect of female body size (s), but we could not detect any significant correlations between age (a) and egg size (e), or between age and clutch size (c): $r_{ae \cdot s}=-0.167$ ($n=18$, $P=0.521$) and -0.148 ($n=8$, $P=0.751$), $r_{ac \cdot s}=-0.142$ ($n=18$, $P=0.587$) and

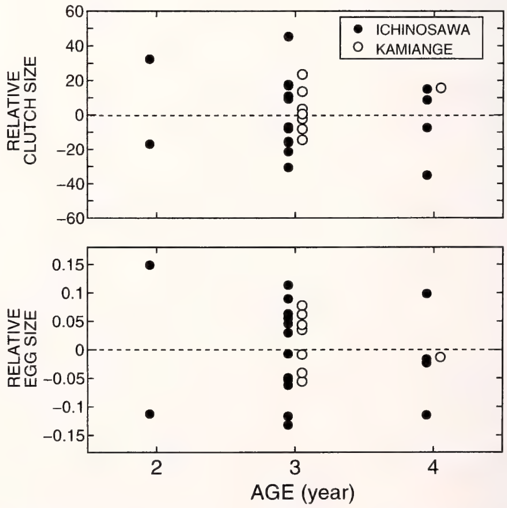

FIG. 6. Relationship between age and clutch parameters adjusted for female body size in *Rana t. tagoi*. Relative clutch parameters were defined as residuals from regressions of clutch parameters against female SVL shown in Figs. 3 and 4.

0.468 (n=8, P=0.290) for the Ichinosawa population and the Kamiange population, respectively.

DISCUSSION

Large egg size and factors affecting egg size

In Japanese anurans, egg size varies greatly from 1.0 to 3.0–4.0 mm in diameter, although reliable quantitative data has not yet been provided for most species (Maeda and Matsui, 1999). This study showed that *R. sakuraii* and *R. t. tagoi* produce small clutches of large eggs, and that *R. sakuraii* is one of the species that lay the largest eggs among Japanese anurans with mean egg diameter being larger than 3.2 mm (Table 1). In salamanders, large egg size, usually accompanying small clutch size, is considered to be a consequence of larval adaptation to lotic environments, such as large prey size and poor productivity of food (Nussbaum, 1985). In *R. sakuraii* and *R. t. tagoi*, unlike salamander larvae, larvae are not carnivorous, and large prey size cannot be a selective force favoring large egg size. In addition to poor productivity, a rapid current itself is considered to be an important factor promoting the evolution of large egg size. In lotic environments, a rapid current prevents larvae from foraging efficiently, and they cannot help largely depending upon yolk to complete metamorphosis.

The two species studied breed in montane streams. Even so, however, *R. t. tagoi* does not spawn in currents of main streams, but breeds in more lentic habitats than those of *R. sakuraii*, such as slowly flowing underground waters on stream banks (Maeda and Matsui, 1999). The selective pressures for large eggs are thus expected to be stronger in *R. sakuraii*, which spawns in main streams, and egg size was actually larger in this species than in *R. t. tagoi* (Table 1 and Fig. 4).

Relationship between female size and egg size

The present study showed that in *R. sakuraii* egg size was not significantly correlated with female body size, but *R. t. tagoi* showed a significant positive correlation between the two parameters (Fig. 4). In amphibian species, positive correlation of these parameters is commonly observed (see Roff, 1992), although a few papers have reported the absence of such correlation. To review the general tendency concerning the relationship between female size and egg size, we gathered relevant published and unpublished data for amphibian species. Data were available for 51 populations representing 28 species (20 for anurans and eight for urodeles: Table 2). Among them, 36 populations (70.6%) showed statistically significant correlations (one-tailed, $P<0.05$), and in all of these populations but that of *Cynops pyrrhogaster*, the correlations were positive. In the remaining 15 populations, egg size did not exhibit statistically significant correlations with female body size. This may possibly be attributable to low statistical power of the test because of the insufficient sizes of samples examined.

In the populations showing significant correlations between the two parameters (n=36: see above), the absolute value of correlation coefficients (r) ranged from 0.21 to 0.87 ($\bar{x}=0.57$, SD=0.18). We calculated the statistical power of the test using the program GPower (Erdfelder et al., 1996), when the correlation was not significant. The power was calculated supposing a large effect (r=0.5) according to Cohen's effect size convention (see Cohen, 1988), because the mean effect size of significant correlations seemed to be high. The result showed that a sufficient power of test ($P>0.8$) required at least 21 specimens, rendering the absence of significant correlation in nine of the 15 populations to be unreliable (Table 2). We thus believe that the non-significant results were reliable only for six populations (11.8%), including the Bonbori population of *R. sakuraii*, and that in most amphibian species the egg size is positively correlated with the female body size.

Evolutionary significance of female size-dependent and size-independent egg sizes

Table 2 shows that relationship between

TABLE 2. Relationship between female body size and egg size in amphibian species. Correlations were tested at the significance level of 0.05 (one-tailed). The power of the test was calculated supposing a large effect (r=0.5) according to Cohen's effect size convention (Cohen, 1988). +/− and NS indicate significant positive/negative correlations and non-significant correlations, respectively. A question mark (?) shows the non-significant result with low power (P<0.8). In *Bombina orientalis* (*), a weighted mean of five years is presented for each population.

Species	r	n	Corr.	Power	Source
Rana sylvatica	0.40	83	+		Berven (1982)
	0.30	87	+		
R. temporaria	0.87	16	+		Ryser (1988)
	0.29	126	+		Gibbons and McCarthy (1986)
R. catesbeiana	0.00	23	NS	0.85	Woodward (1987a)
	0.08	14	NS?	0.65	Howard (1978)
	0.39	10	NS?	0.51	
R. japonica	0.73	31	+		Kuramoto (1978)
R. tsushimensis	0.64	38	+		Kuramoto (1978)
R. tagoi	0.10	21	NS	0.82	Kuramoto (1978)
	0.64	34	+		This study
	0.77	20	+		
R. sakuraii	0.20	41	NS	0.98	This study
	0.36	15	NS?	0.68	
R. nigromaculata	0.37	31	+		Kuramoto (1978)
	0.78	11	+		Shimoyama (1993)
R. porosa brevipoda	0.21	38	NS	0.97	Kuramoto (1978)
R. limnochalis	0.23	19	NS?	0.78	Kuramoto (1978)
Bufo calamita	0.81	10	+		Banks and Beebee (1986)
	0.48	41	+		Tejedo (1992)
B. viridis	0.36	16	NS?	0.71	Jørgensen (1984)
B. woodhousei	0.61	35	+		Woodward (1987a)
Hyla japonica	0.04	32	NS	0.94	Kuramoto (1978)
	0.41	110	+		Ichiyanagi (unpubl.)
Pseudacris triseriata	0.50	16	+		Pettus and Angelton (1967)
	0.46	31	+		
Microhyla ornata	0.40	27	+		Matsui and Ota (1984)
Rhacophorus schlegelii	0.15	9	NS?	0.47	Kuramoto (1978)
Buergeria buergeri	0.84	10	+		Kuramoto (1978)
	0.75	50	+		Fukuyama and Kusano (1989)
*Bombina orientalis**	0.21	143	+		Kaplan and King (1997)
	0.28	731	+		
Scaphiopus couchi	0.34	28	+		Woodward (1987b)
Ambystoma talpoideum	0.40	262	+		Semlitsch (1985)
A. maculatum	0.44	17	+		Woodward (1982)
	0.29	16	NS?	0.71	
Hynobius nigrescence	0.74	17	+		Takahashi and Iwasawa (1989)
	0.52	14	+		
	0.40	17	NS?	0.73	
	0.58	91	+		
	0.64	25	+		
	0.55	17	+		
H. tokyoensis	0.55	34	+		Kusano (1982)
	0.80	25	+		Kusano and Hayase (unpubl.)
	0.70	13	+		
H. tsuensis	0.31	10	NS?	0.51	Kuramoto (1978)
H. kimurae	0.66	15	+		Misawa and Matsui (1997)
	0.72	15	+		

TABLE 2. —Extended.

Onychodactylus japonicus	0.68	23			Kuramoto (1978)
Cynops pyrrhogaster	−0.43	23	–		Kuramoto (1978)
	−0.20	29	NS	0.92	Kusano (unpubl.)

female size and egg size varies not only between species, but also between conspecific populations, such as those belonging to *R. tagoi* or *Hyla japonica*. Smith and Fretwell's (1974) standard model of size-number trade-off predicts that optimal offspring size does not depend on the resource status (corresponding to body size) of females, and that females should produce eggs of a constant size, irrespective of their body size. Then, why do large females actually tend to produce large offspring in most populations or species of amphibians?

Two different models have been presented to explain the evolution of such a positive correlation (see Roff, 1992). Parker and Begon (1986) demonstrated that a positive correlation between female size and offspring size becomes adaptive only if the density of larvae competing with sibs or non-sibs within an oviposition site affects larval success. On the other hand, McGinley (1989) proposed a different model that showed an occurrence of positive correlation when larval survival increases with clutch size due to, e.g., satiation of predators. The situation postulated by McGinley may be uncommon in amphibians, but sib and non-sib competition easily occurs in larvae of a variety of amphibian species, especially those breeding in closed narrow waters. We believe that Parker and Begon's model can be applied to female size-dependent egg size in amphibians.

As mentioned above, *R. t. tagoi* breeds in slowly flowing underground waters on the stream banks. Under such relatively lentic conditions, hatchlings may be able to forage, although they can complete metamorphosis without food (Iwasawa, 1971). If larval foraging occurs in the closed narrow underground waters, sib or non-sib competition must become severe. Such a situation would favor female size-dependent egg size according to the prediction by Parker and Begon's model.

By contrast, *R. sakuraii* breeds in rapid currents of montane streams, and such lotic environments prevent hatchlings from foraging on the stream bottom. Hatchlings from large eggs have a large amount of yolk. They burrow into gravel at the bottom of the stream just after hatching and develop within the gravel layer (Kusano, 1996). Since they complete metamorphosis without foraging by utilizing only their own yolk, a density-dependent process does not occur between larvae. Lack of competition between larvae may promote the evolution of female size-independent egg size in *R. sakuraii*, as Smith and Fretwell (1974) predicted. Thus, differences in breeding microenvironments between the two closely related species might have lead to the different relationships between female size and egg size.

For *R. t. tagoi*, Kuramoto (1978), on the basis of data from a population in Fukuoka, Kyushu, reported absence of significant correlation between female size and egg size (Table 2). The population studied by Kuramoto is more than 800 km WSW of the Kanto populations studied here. Considering that *R. t. tagoi* shows a variety of breeding habits (Maeda and Matsui, 1999), it is probable that the Fukuoka population of *R. t. tagoi* breeds in more lotic environments, although we have little information on the breeding habits of this population.

In addition to larval competition with sibs or non-sibs, clutch frequency may also affect the optimal egg size in relation to female size. Parker and Begon (1986) predicted that when females can divide reproductive resources into several clutches, the correlation between female size and egg size becomes weak. We thus expect that no correlation occurs more frequently in multiple-clutch species (or populations) than in single-clutch ones, and the data of Table 2 seem to agree with this expectation. For example, among 18 Japanese species listed in Table 2, five species are known to produce multiple clutches in a season: *R. catesbeiana, R. porosa brevipoda, R. limnocharis, Hyla japonica*, and *Cynops pyrrhogaster* (Maeda and Matsui, 1999; Ichiyanagi, unpubl.; Hayashi, 1996). None of these species exhibits significant positive correlation between female size and egg size, although statistical power is not sufficient for *R. limnocharis* due to small sample size.

At present, information regarding variation in the relationship between female size and egg size in amphibians is still too insufficient to discuss its evolutionary relevances in detail. Accumulation of additional data is thus much desired.

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Site Selection by *Hynobius tokyoensis* for Breeding in a Stream

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Abstract: A breeding site of the salamander *Hynobius tokyoensis* in a flowing stream was surveyed in the Yamanaka District of Miura Peninsula, central Japan, from March to April 2000, and from February to March 2002. The numbers of males, females, and egg sac pairs found in the stream were 32, three, and 31 in 2000, and 27, three, and 32 in 2002, respectively. The results of field observations suggest that the salamander prefers hollows under broader stones in the stream for breeding and/or spawning, presumably in order to avoid accidental washing away of egg sacs.

Key words: Salamander, *Hynobius tokyoensis*, Breeding site selection, Running water

INTRODUCTION

The jelly surrounding amphibian eggs is highly susceptible to desiccation, freezing, and high temperature, so the survival rate of the eggs is directly influenced by changes in the conditions of their surroundings (Duellman and Trueb, 1986). Several species of frogs and salamanders have been shown to select spawning sites suitable for optimal development of their eggs and for minimization of the risk of egg loss (Duellman and Trueb, 1986; Stebbins and Cohen, 1995).

In the Asiatic hynobiid salamanders, environmental properties of spawning sites differ among the species (Sato, 1943), but data on the environmental factors governing the spawning site selection are available for only one species, *Hynobius nebulosus*, which uses deep cavities under water as sites for both mating and spawning (Tanaka, 1992). Moreover, the proximal causes for such site selection have never been investigated.

Of the Asiatic hynobiid salamanders, *H. retardatus* and *H. nigrescens* exhibit a common feature in the breeding site environment: at their breeding sites, the water temperature is more stable than adjacent portions of same water body and seldom drops low enough to freeze the eggs (Sato, 1989; Akita, 2000). It is therefore probable that in these and other hynobiid salamanders, the major significance of the spawning site selection lies in the minimization of egg mortality.

I have investigated the breeding environment favored by *H. tokyoensis* in the light of avoidance of egg loss. This salamander is distributed in hilly areas of the Kanto District and Fukushima Prefecture, and usually breeds in early spring in ponds, puddles, paddy fields, roadside gutters, and small mountain streams

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(Sato, 1943; Kusano and Miyashita, 1984; Kakegawa et al., 1989; Kusano and Kawakami, 1999). The egg sacs of *H. tokyoensis* are usually attached to twigs, litter, and logs in still water (Kusano and Kawakami, 1999), and under stones in running water (Kakegawa et al., 1989).

In stream breeding, the washing away of eggs may constitute a major cause of egg mortality. This may possibly be a reason why stream breeding salamanders generally attach their eggs beneath stones (Kakegawa et al., 1989; Petranka, 1998), because such sites may help prevent the eggs from being washed away. However, how stones are selected for breeding, and what effects this selection actually has on the protection of eggs, have remained unexamined.

METHODS

Study site

The study site was located at Yamanaka (35°17'N, 139°38'E), Yokosuka City, Kanagawa Prefecture, approximately 30 km southwest of Metropolitan Tokyo. This area, comprising a small hill (110 m asl) with three small streams, is a well-known habitat of *H. tokyoensis* (Shibata, 1973; Kaneda and Ohno, 1998). Field observations were done in the smallest valley, which has a short first-order stream: the distance from the stream head to the confluence with a second-order stream was approximately 40 m. The stream was shaded by broad-leaved trees, and its bed followed a moderate gradient with bedrock exposed in places and a number of scattered stones that are larger than fist size.

The amount of litter, dead branches, and mud accumulated in the stream channel was very small in 2000. In 2002, however, a portion of the channel was covered by mud that had been washed down from the hill. Some of the stones in the channel observed in 2000 were thus buried in the mud in 2002.

The width of the channel ranged 1–2 m. During the winter season, the stream was greatly reduced in width and partially exposed due to low precipitation. Nevertheless, holes under cascades and hollows under stones were still filled with water. In spring, the running water body expanded over the channel due to an increase in precipitation, and this state continued until the next winter drought. The width of this stream, therefore, greatly varied annually, from 40 to 200 cm.

The numbers of individuals and deposited egg sacs

Field surveys were made over five days between 2 March and 6 April 2000, and over 33 days between 16 February and 28 March 2002. Individuals and egg sacs beneath the stones were searched for and counted. Points at which the individuals were captured and locations at which the egg sacs were found in the stream were recorded every day during the study. Positions of stones to which the eggs were attached were recorded on simple diagrams to avoid double counting. Captured individuals were marked by toe clipping for identification and were released.

Microenvironments of spawning sites and sites for males

Microenvironments were compared between sites with and without males, and also between sites with and without egg sacs. During the preliminary field observations conducted in the spring of 1998 and 1999, both males and egg sacs were usually found in hollows formed between undersides of the stones and the streambed. In this study, therefore, I measured the area of the underside of each movable stone larger than 150 mm in length covering a hollow on the streambed, on 8 March 2000, and 16 February 2002. When two stones were stuck together, they were treated as a single stone. For expediency, each was estimated as a quadrilateral area using the longest length and width of the stones as seen from above. I also measured the water depth in the hollows under these stones on 8 and 16 March 2000 and 16 February 2002, and the velocity of the water running around each of the stones on 16 February and 27 and 28 March 2002.

When the water depth in the hollow was first measured on 8 March 2000, there was no running water in the stream channel, but each hollow still had water, enabling me to take its depth. At each site, the stone was removed, and the depth was measured at the deepest part of the hollow. The stone was then carefully replaced immediately.

The velocity of water was quantified by measuring the time needed for a styrofoam ball (5 mm in diameter) to be carried from the upstream edge to the downstream edge of the stone. If currents measured on each side of a stone differed, the faster value was adopted. Because of the rainfall from the evening of 27 to the morning of 28 March, the water level of the stream was slightly higher on the second day.

Stone size selection by salamanders

Bootstrap sampling (Manly, 1997) of 10000 replications was used. The mean of the underside areas was consistently greater, though not statistically significant, for stones with egg sacs than for those with males. Therefore, data for the underside areas of stones, and the numbers of stones with and without egg sacs in each year, were subjected to this analysis.

The number of egg sacs washed away from original spawning sites

To determine the exact number of egg sacs naturally washed away by the running water, the number of egg sacs accidentally removed from the stones at the time of their measurements was excluded from that of egg sacs lost during the study period. The number of egg sacs was counted every day during the study period in 2002.

Territoriality of males

The numbers of stones with no males, a single male, and more than one male underneath were counted and their ratios (%) were calculated for each observation day. These values were then compared with values expected on the basis of probability of binominal distributions. This was to estimate the degree of agonistic interaction (Umesao, 1949). The probability of binominal distributions was calculated following:

$$P(x) = {}_{N}C_{i} \cdot p^{i} \cdot (1-p)^{N-i}$$

where N is the number of males hiding under a stone per day, i is the expected number of males hiding under the stone, and p is the probability of a male hiding under a particular stone.

RESULTS

The number of individuals and egg sacs found

The area and depth were measured for 71 sites in 2000, and 55 in 2002. A total of 32 males were found under 24 stones in 2000, and 27 males under 17 stones in 2002. The number of females found in each year was no more than three: these were found under three separate stones in both years. A total of 62 egg sacs (31 pairs) were found under 14 stones in 2000, and 64 egg sacs (32 pairs) under 14 stones in 2002 (Fig. 1). These egg sacs were attached to the undersides of stones that covered hollows, or to the surfaces of exposed bedrock on the floor of hollows, or to the surfaces of small stones and twigs within hollows (Fig. 2).

Features of hollows used for breeding

The underside areas of stones in the stream had a range of 180–3570 cm^2 ($\bar{x}\pm SD=1039.2\pm 780.9$, $n=71$) in 2000, and 192–3570 cm^2 ($\bar{x}\pm SD=1002.4\pm 885.9$, $n=55$) in 2002. Areas of stones with males had a range of 216–3570 cm^2 ($\bar{x}\pm SD=1309.0\pm 903.0$, $n=24$) in 2000, and 210–3570 cm^2 ($\bar{x}\pm SD=1480.0\pm 998.6$, $n=17$) in 2002, and those of stones with egg sacs had a range of 400–3570 cm^2 ($\bar{x}\pm SD=1771.5\pm 976.0$, $n=14$) in 2000, and 210–3570 cm^2 ($\bar{x}\pm SD=1554.2\pm 1192.6$, $n=14$) in 2002 (Fig. 3). There was no significant difference in this measurement between stones with males in 2000 and 2002 (U–test, $z=-0.61$, $P>0.5$); nor between stones with egg sacs in 2000 and 2002 (U–test, $z=0.73$, $P>0.1$). The underside areas

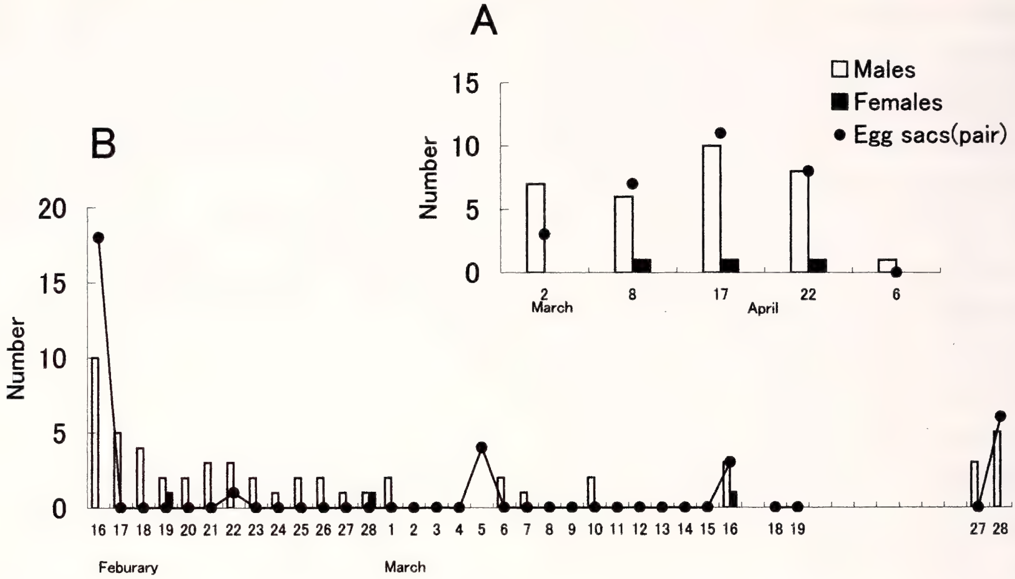

FIG. 1. The numbers of newly found individuals and egg sacs of *Hynobius tokyoensis* during the study periods in 2000 (A) and 2002 (B).

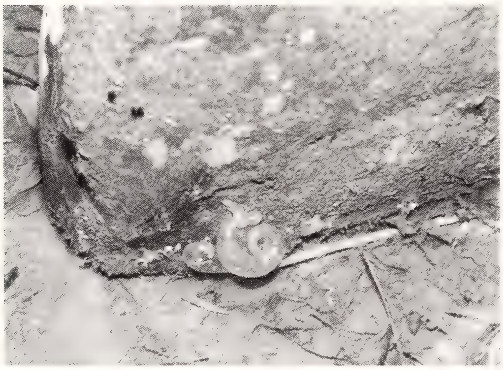

FIG. 2. Egg sacs of *Hynobius tokyoensis* attached to the underside of a stone.

of stones with males were significantly broader than those of stones without males in both years (U–test: z=1.83, P<0.05 in 2000; z=2.70, P<0.01 in 2002). Likewise, underside areas of stones with egg sacs were significantly broader than those of stones without egg sacs in both 2000 (U–test: z=3.84, P<0.01) and 2002 (z=3.13, P<0.01). In contrast, there were no significant differences in the area between stones with egg sacs and those with males (U–test: z=–1.61, P>0.05 in 2000; z=0.44, P>0.05 in 2002), although the mean of areas of stones with egg sacs was consistently greater than that of stones with males.

Larger stones were used by males in both years with significantly higher frequency (Spearman rank correlation coefficient test, $r_s=0.58$, $P<0.01$, n=24 in 2000; $r_s=0.61$, $P<0.05$, n=17 in 2002). However, male body size (SVL) did not correlate with the area ($r^2=0.18$, $P>0.1$, n=32 in 2000; $r^2=0.21$, $P>0.1$, n=27 in 2002). The number of egg sacs deposited in each hollow was marginally significantly correlated with the underside area of the stone in 2000 ($r^2=0.47$, $P=0.05$, n=14), but not in 2002 ($r^2=0.23$, $P>0.1$, n=14).

The water depth in the hollows had a range of 0.0–6.0 cm (x̄±SD=1.2±1.4, n=71) on 8 March 2000, 0.0–6.0 cm (x̄±SD=1.8±1.6, n=71) on 16 March 2000, and 0.0–6.5 cm (x̄±SD=1.53±1.1, n=55) on 16 February 2002. The depth of the hollows with males had a range of 0.0–5.0 cm (x̄±SD=2.6±2.1, n=6) on 8 March 2000, 1.5–6.0 cm (x̄±SD=3.2±1.8, n=11) on 17 March 2000, and 0.0–4.0 cm (x̄±SD=2.4±0.7, n=9) on 16 February 2002; the depth of the hollow with egg sacs had a range of 0.0–5.0 cm (x̄±SD=3.0±2.1, n=5) on 8 March 2000, 1.0–6.0 cm (x̄±SD=3.4±1.7, n=8) on 17 March

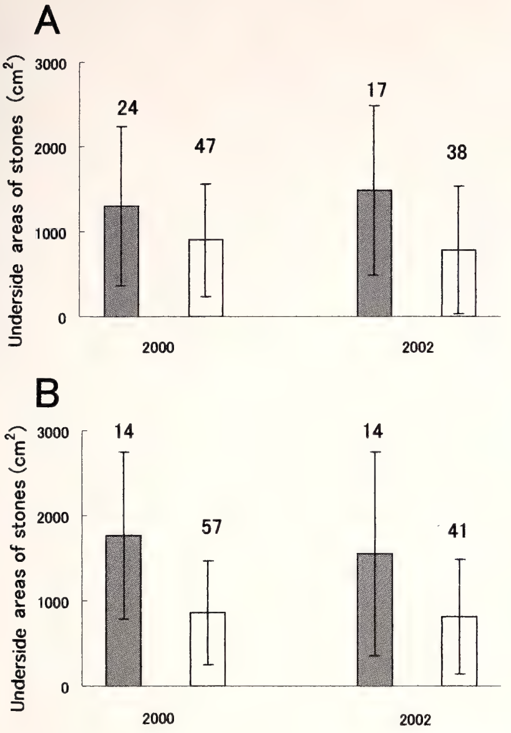

FIG. 3. Mean underside areas of stones with (filled squares) and without (open squares) males (A) and egg sac pairs (B). Vertical bars indicate ranges of standard deviations from means. Numerals above bars denote sample size.

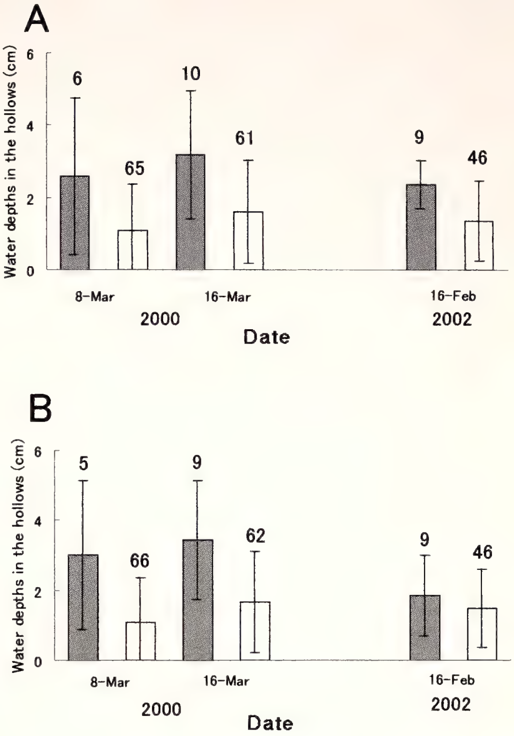

FIG. 4. Mean water depths of hollows with (filled squares) and without (open squares) males (A) and egg sac pairs (B). Vertical bars indicate ranges of standard deviations from means. Numerals above bars denote sample size.

2000, and 0.0–4.0 cm ($\bar{x}\pm SD=1.3\pm1.1$, $n=9$) on 16 February 2002 (Fig. 4).

Water depths of hollows with males were consistently significantly greater than those of hollows without males (U–test: $z=1.76$, $P<0.05$, on 8 March 2000; $z=3.22$, $P<0.01$ on 17 March 2000; $z=3.34$, $P<0.01$ on 16 February 2002). Likewise, the depths of hollows with egg sacs were consistently significantly greater than those of the hollows without egg sacs (U–test: $z=2.05$, $P<0.01$ on 8 March 2000; $z=2.82$, $P<0.01$ on 17 March 2000; $z=2.06$, $P<0.05$ on 16 February 2002).

The velocity of running water around the stones had a range of 0.0–4.0 cm/sec ($\bar{x}\pm SD=0.09\pm0.92$, $n=55$) on 16 February, 0.0–6.0 cm/sec ($\bar{x}\pm SD=1.62\pm1.20$, $n=55$) on 27 March, and 9.0–30.0 cm/sec ($\bar{x}\pm SD=17.33\pm5.53$, $n=55$) on 28 March 2002. The water velocity around stones with males had a range of 0.0–4.0 cm/sec ($\bar{x}\pm SD=1.22\pm1.20$, $n=9$) on 16 February, 1.0–3.0 cm/sec ($\bar{x}\pm SD=1.83\pm1.04$, $n=3$) on 27 March, and 10.0–30.0 cm/sec ($\bar{x}\pm SD=20.2\pm7.91$, $n=5$) on 28 March 2002 (Fig. 5). Newly deposited egg sacs were not found on 16 February or 27 March. The water velocity around stones with newly deposited egg sacs on 28 March had a range of 10.0–21.0 cm/sec ($\bar{x}\pm SD=16.6\pm4.9$, $n=5$) (Fig. 5). There was no significant difference in velocity of surrounding water between stones with and without males (U–test: $z=0.89$, $P>0.1$ on 16 February; $z=0.74$, $P>0.1$ on 27 March; $z=1.06$, $P>0.1$, on 28 March); between stones with and without newly deposited egg sacs on 28 March ($z=-0.51$, $P>0.5$); or between stones with males and with egg sacs on 28 March ($z=1.04$, $P>0.1$).

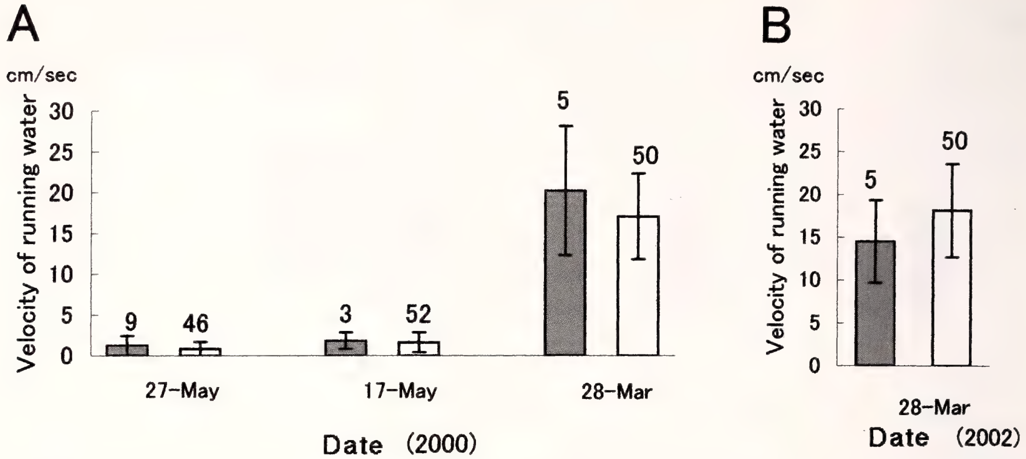

FIG. 5. Mean velocity of running water around stones with (filled squares) and without (open squares) males (A) and egg sac pairs (B). Vertical bars indicate ranges of standard deviations from means. Numerals above bars denote sample size.

Stone size selection by salamanders

Selectivity for broad stones by females tested by bootstrapping was statistically significant in 2000 ($P<0.05$), but not in 2002 ($P>0.05$).

The number of egg sacs washed away

There were no egg sacs washed away after the rainfall on 17 and 27 February or on 6 and 27 March 2002 despite a prominent rise in the stream water level at those times.

The number of males per stone

Most stones with males were occupied by single males. The only exceptions were two stones which each had two males underneath on 22 March 2000 and 16 February 2002. Except for these cases, observed frequency of stones with single males always exceeded expected values (Table 1).

DISCUSSION

Kusano and Kawakami (1999) argued that *H. tokyoensis* prefers to breed in shallow waters of small ponds or puddles to avoid aquatic predators, such as fishes, that require deeper water for their habitats. This may explain why this salamander breeds in the present stream, where the water depth remains shallow even after a rainfall.

The hollows used for breeding exhibited several common features, such as coverage by relatively broad stones, and locations in relatively deep positions in the stream. This suggests that the salamanders select particular hollows over the others for spawning to avoid accidental washing away of egg sacs from hollows, although the results of bootstrapping for data from 2002 failed to support selection of broader stones by females. Results of the bootstrap in 2002 may be attributable to a shortage of available hollows under broad stones due to accumulation of mud washed down from the hill in this and previous years (see above).

There were no egg sacs actually washed away during study period in 2002. This may reflect the effectiveness of depositing egg sacs under broad stones to avoid their being washed away.

Water depth of hollows used by the salamanders was significantly greater than that of unused hollows. This seems to contradict the data of Kusano and Kawakami (1999), which suggest a possible preference of the salamanders for shallower sites for spawning. Further work is needed to come to a conclusion on this issue.

In the stream, the frequency of stones with only a single male almost consistently exceeded

TABLE 1. Comparisons between observed (O, in %) and expected frequencies (E, probability on an assumption of binominal distribution, in %) of stones without males, and with one or more than one male on each day. Data for days on which fewer than two males were observed are excluded.

	2000. Mar.								2002. Feb.									
	2		8		17		22		16		17		18		19		20	
No. of stones with males	7		6		10		7		9		5		4		2		2	
No. of males found	7		6		10		8		10		5		4		2		2	
Mean male no./stone with males	1.00		1.00		1.00		1.14		1.11		1.00		1.00		1.00		1.00	
No. of males per stone	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O
0	90.50	90.10	91.80	91.50	86.70	85.90	90.50	90.10	83.24	83.64	91.23	90.91	92.92	92.73	96.40	96.36	96.40	96.36
1	9.10	9.90	7.90	8.50	12.40	14.10	9.10	8.50	15.41	14.55	8.45	9.09	6.88	7.27	3.57	3.64	3.57	3.64
>1	0.40	0.00	0.30	0.00	0.90	0.00	0.40	1.40	1.35	1.82	0.32	0.00	0.19	0.00	0.03	0.00	0.03	0.00

											Mar.											
	21		22		23		25		26		1		6		10		16		27		28	
No. of stones with males	3		3		2		2		2		2		2		2		3		3		5	
No. of males found	3		3		2		2		2		2		2		2		3		3		5	
Mean male no./stone with males	1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00	
No. of males per stone	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O	E	O
0	94.64	94.55	94.64	94.55	96.40	96.36	96.40	96.36	96.40	96.36	96.40	96.36	96.40	96.36	96.40	96.36	94.64	94.55	94.64	94.55	91.23	90.91
1	5.26	5.45	5.26	5.45	3.57	3.64	3.57	3.64	3.57	3.64	3.57	3.64	3.57	3.64	3.57	3.64	5.26	5.45	5.26	5.45	8.45	9.09
>1	0.10	0.00	0.10	0.00	0.03	0.00	0.03	0.00	0.03	0.00	0.03	0.00	0.03	0.00	0.03	0.00	0.10	0.00	0.10	0.00	0.32	0.00

the expected value. This strongly suggests that the males prefer to stay alone. Kusano and Kawakami (1999) reported that males of this salamander in ponds aggressively guarded from other males particular sites that were seemingly suitable for spawning. It is likely that in the present stream males guarded their hollows from others through aggressive interactions. Considering that the mean of areas of stones with males was consistently smaller than that of stones with egg sacs, such guarding of hollows under broader stones by males may lead to a form of sexual selection.

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First Nesting Record of the Leatherback Turtle, *Dermochelys coriacea*, in Japan

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Abstract: Nesting of the leatherback turtle *Dermochelys coriacea* in Japan was confirmed for the first time on the basis of two nests, each containing a clutch of eggs, found on Katoku Beach of Amami-oshima Island, central Ryukyus. There were no indications of development in eggs obtained from them. These nests were likely to have been made by the same individual.

Key words: leatherback turtle; *Dermochelys coriacea*; nesting; Ryukyu Archipelago; Japan

The leatherback turtle, *Dermochelys coriacea*, is the largest species of the extant marine turtles, and is known from almost the entire area of the Pacific, Indian, and Atlantic Oceans (Marquez, 1990). The major nesting grounds of the Pacific populations of this species are confined to a few small areas in the tropics, such as Trengganu in Malaysia (Fitter, 1961), northwestern New Guinea (Salm, 1981), the Solomon Islands (McKeown, 1977), and the western coast of central America (Pritchard and Trebbau, 1984). Although the leatherback is sometimes found in waters adjacent to Japan, nesting of this species has never been recorded with substantial evidence in Japan (Kamezaki, 1994). We report discovery of two nests of the leatherback turtle from a beach on Amami-oshima Island, southwestern Japan.

At Katoku Beach of Amami-oshima Island (Fig. 1), the emergence of one leatherback turtle was noticed by inhabitants at 14:00 on 28 June 2002. This individual, estimated to be approximately 1.8 m in straight carapace length on the basis of photographs (Fig. 2), dug an egg chamber, deposited eggs in it, and returned to the sea. Examination of the nest on 30 June 2002 yielded a total of 20 apparently normal eggs and 14 yokeless eggs. The mean diameter of the normal eggs was 53.0 mm (SD=0.896; range: 51.0–55.0 mm).

On 11 July 2002, broad tracks and an indentation were found on the same beach, and a nest found just beneath the latter contained 66 apparently normal eggs and 18 yokeless eggs. The mean diameter of the normal eggs was 54.4 mm (SD=0.964; 51.5–56.1 mm).

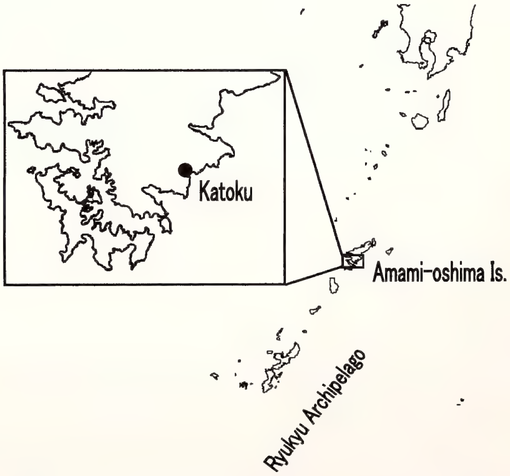

FIG. 1. Map showing location of the nesting site of the leatherback turtle, *Dermochelys coriacea*, on Amami-oshima Island, Ryukyu Archipelago, Japan.

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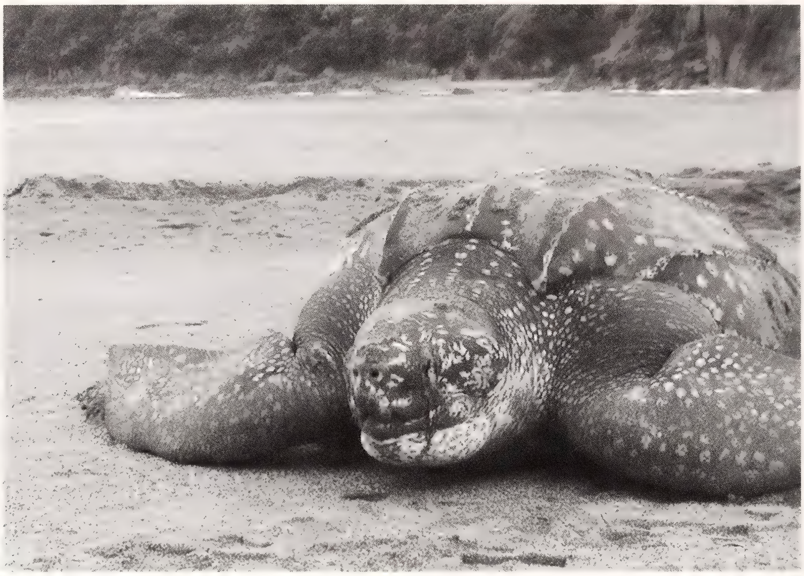

FIG. 2. The nesting leatherback turtle, *Dermochelys coriacea*, on Katoku Beach, Amami-oshima Island. Photographed on 28 July 2002 by Yoichi Baba.

The 20 apparently normal eggs from the first nest were moved to a styroform box for incubation under permission from the Fisheries Agency of Japan. However, no external signs of post-ovipositional development (Chan, 1989) were observed thereafter. One egg, opened on the eighth day, had a blastodisc, but with no indications of development from the ovipositional stage (Miller, 1985).

In the Ryukyu Archipelago, where Amami-oshima Island is located, three species of marine turtles, the loggerhead turtle *Caretta caretta*, the green turtle *Chelonia mydas*, and the hawksbill turtle *Eretmochelys imbricata*, are known to nest (Kamezaki, 1989). One case of nesting of the leatherback turtle was once mentioned by an inhabitant on another island (Zamamijima Island of the Okinawa Group) but without any substantial evidence (H. Ota, private communication). The observation made on 28 June 2002 thus offers the first confirmed record of nesting of this species in Japan.

The number of eggs found in the first nest (34 including the abnormal eggs) was much smaller than the ordinary size of a nest clutch in the leatherback turtle (Marquez, 1990). This, along with the extreme softness of the sand covering that nest, suggests that a large proportion of eggs originally laid therein had already been poached by someone when the nest was examined by us on 30 June.

With respect to the second nest, the emerging female was not observed. Even so, however, there is no doubt that this nest also belonged to the leatherback turtle, because size of its eggs (51.0–56.1 mm) was much larger than that of eggs of the other marine turtles nesting in the Ryukyus (34–46 mm: Kamezaki, 1987), and fell within the known range of egg sizes in the leatherback turtle (45.6–59.2 mm: Marquez, 1990). These two nests are likely to have been made by the same individual, considering that they were found on the same beach with an interval (13 days) close to the known renesting intervals in this species (9–11 days: Pritchard and Trebbau, 1984).

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Titles of Papers Presented at the 41st Annual Meeting of the Herpetological Society of Japan (5-6 October 2002 at Hokkaido Tokai University, Sapporo)

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3. Effects of temperature on the life-cycle of the salamander, *Hynobius nebulosus*. Yuko Murakami
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5. Enviromental changes in the larval habitat of *Hynobius kimurae* in Hachioji, Tokyo. Yasuchika Misawa and Masafumi Matsui
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7. Molecular cytogenetic analysis of highly repetitive DNA in four hynobiid salamanders, with comments on their phylogenetic relationships. Yoko Hasegawa, Ayumi Yamamoto, Eriko Inada, Chikako Ikebe, Sei-ichi Kohno, Guanfu Wu, Xiaomao Zeng, and Masaki Kuro-o
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11. Food habits of the newt *Cynops pyrrhogaster* in high mountain marshes. Sadao Ihara
12. Breeding migration of *Echinotriton andersoni*. Satoshi Tanaka

[Frogs and toads]

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HIRATE, K. 2000. Species Composition, Sex Ratio, Size Distribution, and Seasonal Fluctuation of the Sea Turtle Assemblage in the Eastern Off-shore of the Central Part of Okinawajima. Unpublished master's thesis. University of the Ryukyus, Nishihara. (in Japanese)

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TAKENAKA, T. 2000. Extinction of the naturalized freshwater turtle in Chichijima-Island

of Ogasawara (Bonin) Islands, South Japan. Bull. Herpetol. Soc. Japan 2000: 4-7. (in Japanese with English abstract)
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APPENDIX

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Geoemyda japonica: Okinawajima, Okinawa Pref., Japan, KUZ R36720, NSMT H02083-02086; Kumejima, Okinawa Pref., Japan, KUZ R36721, OMNH-R3334. G. spengleri: Vietnam, NSMT H9999, ...

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$\bar{x}$ (mean), n (sample size), N (chromosome number, but see below for the use to refer to "north latitude"), no. (number), yr (year[s]), mo (month[s]), wk (week[s]), h (hour[s]), min (minute[s]), s (second[s]), P (probability), df (degrees of freedom), SD (standard deviation), SE (standard error), NS (not significant), l (liter), kg (kilogram), g (gram), m (meter), cm (centimeter), mm (millimeter), μm (micron), C (degrees Celsius, not °C), asl (above sea level; given as, e.g., 100 m asl), °, ', and " (degrees, minutes, and seconds in geography, respectively), N, S, E, and W (north and south latitudes, and east and west longitudes, respectively, but only when preceded by values with appropriate geographical units; e.g., 15°25'N, 121°43'E).

In figures and tables, names of months can be abbreviated as: Jan (January), Feb (February), Mar (March), Apr (April), May (May), Jun (June), Jul (July), Aug (August), Sep (September), Oct (October), Nov (November), Dec (December). In the text, however, they should be fully spelled.

Other abbreviations, such as SVL (snout-vent length), Myr (million years), and MyrBP (million years before present), can also be used, but **define them at first usage**. Do not use symbols, ♂ and ♀, for male and female, respectively.

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CURRENT HERPETOLOGY

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